Contribution of Sensory Afferents and Sympathetic Efferents to Joint Injury in Experimental Arthritis

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We used pharmacological and surgical methods to determine the contribution of several neural components to joint injury in rats with adjuvant-induced arthritis. Both neonatal administration of capsaicin, which eliminates small-diameter afferents, and peripheral sympathectomy, which depletes catecholamines, attenuated joint injury. In contrast, the arthritis was more severe in spontaneously hypertensive rats, which have increased sympathetic tone. To address the contribution of the central vs peripheral afferent terminal selectively, a group of rats underwent unilateral dorsal rhizotomy. These rats developed a more severe arthritis in the deafferented limb. The increase in arthritic severity produced by dorsal rhizotomy could be reduced by prior sympathectomy or, less effectively, by prior treatment with capsaicin. The latter observation suggests that large-diameter afferents that are cut during dorsal rhizotomy also influence inflammation. Finally, intracerebroventricular injection of morphine attenuated the severity of arthritis, possibly through activation of bulbospinal sympathoinhibitory circuits. Taken together, these data indicate that no one class of nerve fiber is wholly responsible for the neurogenic component of inflammation in experimental arthritis but that large- and small-diameter afferents, sympathetic efferents, and CNS circuits that modulate those fiber systems all influence the severity of joint injury in arthritic rats.

Lewis (1927, 1941), in his classic axon reflex experiments, suggested that primary afferent nociceptors contribute to inflammation. His studies attributed the cutaneous wheal and flare response to substances released from the peripheral terminals of nociceptive afferents. A candidate for this neurogenic inflammatory mediator is the undecapeptide neurotransmitter substance P (SP). Found in small-diameter, predominantly unmyelinated afferent axons (Cuello et al., 1978; Hökfelt et al., 1975), SP is transported from dorsal root ganglion cells to peripheral sensory endings, where it is stored in a vesicular compartment and can be released by electrical stimulation (Bill et al., 1979; Olgart et al., 1977). Most important, many of the physiological changes associated with acute inflammation can be produced either by electrically stimulating peripheral nerves

at the same intensities that release SP into peripheral tissue (Bill et al., 1979; Brodin et al., 1981; Jancso et al., 1967) or by applying SP directly (Foreman and Jordan, 1983; Lembeck and Holzer, 1979; Lembeck et al., 1977). Finally, destruction of unmyelinated peripheral afferent axons with the neurotoxin capsaicin depletes SP and significantly attenuates the inflammatory response produced by peripheral nerve stimulation (Gamse et al., 1980, 1981) or by injection of noxious substances (Gamse et al., 1980).

A contribution of the PNS to the inflammation in an arthritic rat model has also been established. Courtright and Kuzell (1965) demonstrated that peripheral nerve section attenuated the severity of adjuvant-induced arthritis in the rat. Colpaert et al. (1983) subsequently reported that treatment of rats with capsaicin, either before or after the onset of adjuvant-induced arthritis, significantly reduced the swelling and hyperalgesia of arthritic joints. Recently, we demonstrated that the highest levels of intraneuronally derived SP are found in joints having the most severe arthritis (Levine et al., 1984). Furthermore, infusion of SP into the knee joint (which normally develops only mild arthritis) significantly increased the severity both of inflammation and of joint destruction.

Clinical evidence also indicates that the peripheral limb of the sympathetic nervous system (SNS) contributes to inflammation. For example, reflex sympathetic dystrophy is manifested by pain, marked sympathetic hyperactivity, and inflammation of synovial joints (Kozin et al., 1976). Regional sympathetic blockade with guanethidine (Bonica, 1979; Hanington-Kiff, 1977; Loh and Nathan, 1978) or other sympatholytic agents (Benzon et al., 1980) can reduce the inflammation. We have found that sympathectomy also reduces a reflex neurogenic inflammation that is generated at sites remote from injury (Levine et al., 1985).

Peripheral interaction between primary afferent nociceptors and sympathetic efferents may also increase inflammation. Indeed, there are facilitatory interactions between sympathetic efferents and sensory afferents in neuromas (Devor and Janig, 1981; Scadding, 1981; Wall and Gutnick, 1974) and in heat-sensitive afferent fibers (Roberts and Elardo, 1985). Activation of nociceptive afferents also produces marked increases in the activity of postganglionic sympathetic efferents (Beacham and Perl, 1964; Blinn et al., 1980; Koizumi et al., 1970). Interruption of these interactions may provide prolonged antiinflammatory effects in a variety of disease states.

Taken together, these data indicate that manipulation of the sensory and sympathetic innervation of the joint can modify the severity of inflammation. It is also likely that they contribute to the inflammation and joint injury in arthritis. In this study we have used a variety of surgical and pharmacological manipulations to assess the relative contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis.

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Materials and Methods

The experiments were performed on 250–350 gm male Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) and on 150–250 gm male Wistar-Kyoto spontaneously hypertensive (SHR) and normotensive (WKY) rats. After the appropriate surgical or pharmacological procedures were completed, arthritis was induced by intradermal injection of 0.1 ml of a 10 mg/ml suspension of *Mycobacterium butyricum* in mineral oil (Pearson and Wood, 1959). Arthritic rats showed decreased mobility, presumably a response to their hyperalgesic condition (Dardick et al., 1986). However, they did groom themselves and engage in sexual behavior. Arthritic rats were bedded on soft wood shavings. Food and water were placed within easy reach inside the cages, and all rats were observed to eat and drink.

To evaluate the onset time of arthritis, the rats were examined for the presence of signs of inflammation (i.e., swelling and tenderness) every day. In rats that were otherwise untreated (i.e., had neither surgical nor pharmacological treatment), mild swelling (i.e., grade 1) first appeared 15.2 ± 3.3 d (average $\pm$ SD, $n = 39$ paws) d after injection of the mycobacterium. (Six paws, 13.3%, did not reach a score of 1 during the 28 d period of examination.)

Twenty-eight days after the injection of the adjuvant, we assessed the severity of arthritis by x-raying the rats (Ackerman et al., 1979; Buckland-Wright, 1981; Jamieson et al., 1985). To describe the severity of disease, a "blind" observer (C.H.) evaluated the radiographs using the grading scale of Ackerman et al. (1979), which assesses the following signs of injury: soft tissue swelling, decreased bone density (osteoporosis), narrowing of the joint space (loss of cartilage), destruction of bone (erosions), and formation of periosteal new bone. Radiographic scores derived with this scale correlate well with scores from histological sections of arthritic joints and periarticular tissues (Ackerman et al., 1979). A score of 0–3 was used to grade severity of the radiographic signs of injury. In the nonlesioned arthritic model, the maximum severity of the hindpaw is generally much greater than that of the forepaw. Thus, a score of 3 for the forelimb indicates maximal forelimb damage, even though the absolute damage is less than that seen in a hindlimb that also is scored 3. The radiographic scores were used to assess nervous system influences on the severity of arthritis. The rats were killed on day 28, immediately following radiography.

Treatment with capsaicin

We administered the neurotoxin capsaicin (50 mg/kg, $n = 28$, and 100 mg/kg, $n = 14$, s.c.) in a vehicle of 50% dimethyl sulfoxide (Sigma, St. Louis) and 50% saline to a group of rats on neonatal day 2. This protocol produces a significant depletion of SP in the peripheral nerves and in the joint capsule (Levine et al., 1985) and depletes 90–95% of unmyelinated afferents (Lawson, 1981; Nagy et al., 1983). When these rats weighed 250–350 gm, they were injected with mycobacterium to induce arthritis.

Sympathectomy

We sympathectomized a group of rats by injecting guanethidine (5 mg/d) for 6 weeks. In the rat, this regimen produces depletion of catecholamines by destroying the sympathetic postganglionic neurons without injuring catecholaminergic neurons of the CNS (Burnstock et al., 1971; Jensen-Holm and Juul, 1970). The effect of this chronic guanethidine treatment is thought to result from immunological destruction of postganglionic sympathetics (Manning et al., 1983). The effectiveness of this guanethidine sympathectomy was confirmed with a sensitive radioenzymatic microassay of tissue norepinephrine levels (Da Prada and Zürcher, 1976). We found no detectable amount of norepinephrine in the sciatic or femoral nerves or in the knee or ankle joint capsules, even when measurements were taken a month after the last injection of guanethidine.

Since guanethidine may activate the immune system, another group of rats received a daily subcutaneous injection of another catecholamine depletor, reserpine (0.25 mg/kg/d) starting 2 d before the induction of arthritis. We were able to confirm a significant depletion of peripheral norepinephrine as early as 24 hr after the first daily injection (<5 vs 780 ± 138 pg/ankle capsule, both $n = 4$, $p < 0.002$).

Spontaneously hypertensive rats

We also studied the severity of adjuvant-induced arthritis in a strain of rats having abnormally high activity in the sympathetic nervous system. We used the spontaneously hypertensive rat (SHR/NCr1BR) derived

from the original pedigreed NIH strain that has a tonically increased sympathetic tone (Nilsson and Folkow, 1982; Okamoto et al., 1967). Since there is a marked interstrain variability in the severity of adjuvant-induced arthritis in the rat, the normotensive Wistar-Kyoto rat (WKY/NCr1BR), which also derives from the original pedigreed NIH strain, was used as the control group.

Dorsal rhizotomy

Capsaicin administered to neonatal rats destroys cells of the dorsal root ganglia and thus eliminates both the central and peripheral branches of primary afferent fibers. To selectively eliminate the central connections of joint afferents of all diameters while leaving peripheral connections intact, we subjected 13 rats to a unilateral deafferentation of the hindlimb (i.e., to unilateral dorsal rhizotomy of dorsal roots L₄, L₅, and L₆). A laminectomy was performed under pentobarbital anesthesia. The dura was incised on the midline and reflected laterally to expose the L₄₋₆ dorsal roots as they joined the ganglia. The underlying ventral rootlets were readily identified and avoided. After the appropriate roots were cut, a strip of Gelfilm was placed over the spinal cord, the overlying muscle and skin were sutured, and the animals were allowed to recover. Seven days after surgery we injected mycobacterium to induce arthritis.

Neurological examination confirmed that the rhizotomized limb was completely deafferented but was not paralyzed. The rat was unresponsive to all stimuli applied to the denervated limb, but when the top of the intact contralateral paw was touched to the bottom of a horizontal bar while the rat was being held around the chest, the rat made a bilateral stepping response. This indicates that the ventral root supply to the deafferented limb was intact. To prevent the autotomy behavior that occurs in rhizotomized rats (Basbaum, 1974), and which we found also occurs in rhizotomized arthritic rats, we housed operated male rats with unoperated female rats (Berman and Rodin, 1982).

Since in normal rats the forepaw develops less severe arthritis than the hindpaw, it seemed possible that the forepaw might be more sensitive to changes in the neurogenic component of inflammation. Therefore, a separate group of rats underwent unilateral cervical rhizotomy. After laminectomy, dorsal roots C₅–T₁ were sectioned intradurally. Postoperative recovery was rapid, and, again, by housing operated males with unoperated females, autotomy was avoided.

Mixed lesions

Dorsal rhizotomy nonselectively eliminates the central transmission of both small- and large-diameter afferent inputs. Thus, to assess whether the effect of rhizotomy on arthritic joint injury is specifically mediated by changes in activity of unmyelinated afferents, we repeated the deafferentation study in rats that were given capsaicin at birth. Unilateral dorsal rhizotomy was performed when these rats reached a weight of 250–350 gm. Seven days later, we induced arthritis by injecting the mycobacterium.

We also assessed whether the effects of rhizotomy require an intact sympathetic nervous system. To this end, we first sympathectomized 6 rats with guanethidine as described above. One month after the last injection, we performed a unilateral forelimb deafferentation (i.e., cervical dorsal rhizotomy) in these rats. Seven days later, arthritis was induced by the standard protocol.

Intracerebroventricular administration of morphine

We also tested the possibility that the effects of rhizotomy might be mediated, in part, by an action on the CNS. Since sympathectomy attenuates the severity of adjuvant-induced arthritis (Levine et al., 1985), we reasoned that any manipulation that decreases the activity in sympathetic efferents might reduce the severity of arthritis. Since morphine acts centrally to inhibit the preganglionic sympathetic outflow, we injected morphine into the third ventricle of the brain. This activates descending controls of spinal segmental circuits and inhibits the activity of various spinal neurons, including the sympathetic preganglionic neurons of the thoracic part of the spinal cord (Karoum et al., 1982).

Morphine (15 μ g in 1 μ l of vehicle) was injected every 2 hr for 72 hr via a stereotactically placed, chronically implanted, 22 gauge cannula. One hour after the first injection of morphine, we induced arthritis by injecting mycobacteria into the tail. Control rats were given an intracerebroventricular injection of saline every 2 hr for 72 hr and also made arthritic by injecting adjuvant 1 hr after the first saline dose. The location of the tip of the cannula was confirmed by injection of Trypan blue and histological examination.

Table 1. Degree of joint injury in the hindlimb of arthritic rats with selective lesions of the peripheral nerve

Treatment	n	Radiographic score ^a (%)					p ^b
		0	1	2	3	Mean	
None (controls)	60	5	15	33	47	2.4	
Capsaicin	42	26	12	29	33	1.8	<0.05
Guanethidine	22	76	5	14	5	0.5	<0.001
Reserpine	12	66	17	17	—	0.5	<0.001

^a Radiographic scoring based on the scale of Ackerman et al. (1979). 0 = no effect; 1 = mild effect; 2 = moderate effect; and 3 = severe effect. Values indicate the percentage of rats in a treatment group with that score.

^b Vs no treatment (control) group.

Statistical analyses

All the values in the text and tables are given as means $\pm$ SEM. Catecholamine levels in normal and treated groups were compared using Student's *t* test. Onset of arthritis was defined as being the first day on which a clinical score of 1 or greater was encountered. Operated and nonoperated sides were compared using the paired-sample *t* test. For statistical analysis of radiographic scores, experimental groups were compared using contingency tables and the χ^2 statistic. Where this process yielded inadequate cell frequencies, scores of 0 and 1 and of 2 and 3 were combined to produce 2×2 tables, which were then analyzed using the Cochran-corrected χ^2 statistic. When these 2×2 tables still possessed inadequate cell frequencies, comparisons were made using either a Fisher exact test or a Sign test. All tests were computed as described by Zar (1984), and *p* values are given for the 2-tailed test.

Results

To study the effects of the various neurons in peripheral nerves on the severity of arthritis, we subjected groups of rats to a variety of surgical and pharmacological manipulations of the PNS.

Treatment with capsaicin

The contribution of the small-diameter unmyelinated (C) and thinly myelinated (A-delta) primary afferents to the severity of joint injury was evaluated in capsaicin-treated rats. Prenatal treatment of rats with capsaicin depleted 63% of the SP in the ankle joint capsule (Levine et al., 1984). In the separate group of rats given capsaicin as neonates and later made arthritic, the severity of the hindlimb joint injury, evaluated from x-rays taken on the 28th d after induction of arthritis, was significantly less than in control rats not given capsaicin (see Table 1: 1.8 ± 0.2 vs 2.4 ± 0.1 , $n = 42$ and 60 ; $p < 0.05$, χ^2 test).

Sympathectomy

The contribution of sympathetic innervation of the joint to the severity of joint injury was evaluated in sympathectomized rats. Guanethidine sympathectomy, completed 1 month prior to treating rats with mycobacterium to induce arthritis, significantly decreased the severity of the hindlimb joint injury, evaluated from x-rays taken on the 28th d after induction of arthritis, when compared with control rats (Table 1: 0.5 ± 0.2 vs 2.4 ± 0.1 , $n = 22$ and 60 , $p < 0.001$ by Cochran corrected χ^2 test). A similar attenuation in severity of joint injury was produced by daily treatment with reserpine beginning 2 d before adjuvant injection and continuing throughout the period of observation (Table 1: 0.5 ± 0.2 vs 2.4 ± 0.1 , $n = 12$ and 60 , $p < 0.001$ by Fisher exact test).

As a further test of the contribution of activity in the SNS to the severity of joint injury in adjuvant arthritis, we evaluated the effect of tonically increased sympathetic activity. SHR rats have an increased activity of their sympathetic nervous system with respect to their parent, normotensive strain (the WKYs). The

Table 2. Comparison of degree of joint injury between operated and unoperated forelimbs of arthritic rats

Treatment	n	Limb	Radiographic score ^a (%)					p
			0	1	2	3	Mean	
None (controls)	41	—	37	5	29	29	1.7	
Cervical dorsal rhizotomy	10	Deaff.	10	30	10	50	2.3	<0.002
		Intact	40	—	50	10	1.6	
Cervical dorsal rhizotomy and capsaicin	8	Deaff.	12.5	25	62.5	—	1.6	<0.05
		Intact	62.5	37.5	—	—	0.4	
Cervical dorsal rhizotomy and guanethidine	6	Deaff.	17	33	50	—	1.5	n.s.
		Intact	67	—	33	—	0.8	

Deaff., deafferented; n.s., not significant.

^a See Table 1 for explanation.

severity of the arthritis, at day 28, in the hindlimbs of the SHR rats, in fact, was significantly greater than in the WKY rats (2.4 ± 0.3 vs 0.9 ± 0.3 , both $n = 16$; $p < 0.025$ by Cochran corrected χ^2 test).

Dorsal rhizotomy

The attenuating effects of treatment with capsaicin (Colpaert et al., 1983) are consistent with the hypothesis that the peripheral terminals of sensory afferent fibers contribute to the process of inflammation. In contrast to the attenuation of arthritis seen in rats with peripheral nerve section (Courtright and Kuzell, 1965) or in rats that were neonatally treated with capsaicin, arthritis in the deafferented forelimb (cervical rhizotomy) was significantly worse than in the neurologically intact, contralateral forelimb (see Fig. 1, Table 2: 2.3 ± 0.5 vs 1.6 ± 0.4 , $n = 10$; $p < 0.0016$ by sign test). Importantly, there was no significant difference between the severity of joint injury in the forepaw contralateral to a forelimb rhizotomy and the forepaws of nonlesioned control rats with arthritis (1.6 ± 0.4 vs 1.7 ± 0.2 , $p =$ n.s. by Cochran corrected χ^2). Similarly, the onset of arthritis in the forelimb, as determined by clinical examination, was significantly earlier in the deafferented paw than in either the intact paw of the same rhizotomized animals (14.4 ± 0.6 vs 19.7 ± 1.5 d, $n = 10$; $p < 0.002$ by paired Student's *t* test) or the forelimb of arthritized control (nonoperated) rats (14.4 ± 0.6 vs 20.6 ± 1.0 d, $n = 10$ and 36 ; $p < 0.001$ by Student's *t* test).

In the hindlimb, the onset of arthritis in the deafferented limb (lumbar rhizotomy) was once again significantly earlier than in both the intact contralateral limbs (12.5 ± 0.6 vs 19.1 ± 1.7 d, $n = 12$; $p < 0.001$ by paired Student's *t* test) and the hindlimbs of arthritized control (nonoperated) animals (12.5 ± 0.6 vs 15.2 ± 0.5 d, $n = 12$ and 39 , $p < 0.02$ by Student's *t* test). In contrast to the forelimb, however, the hindlimb in this disease model develops severe arthritis anyway, and so the difference in severity between the deafferented limb and the normal limb was no longer significant by the time of radiographic examination on day 28 (2.7 ± 0.0 vs 2.4 ± 0.0 , $p =$ n.s. by Cochran corrected χ^2 test).

Mixed lesions

Since capsaicin destruction of small-diameter primary afferents decreased the arthritis, it appeared that the increased arthritis produced by rhizotomy might have resulted from enhanced activity in the unmyelinated peripheral afferent terminals of the joint. These are destroyed by capsaicin, but they are intact after rhizotomy. As described above, however, rhizotomy nonselectively severs large- and small-diameter afferents in the dorsal

Figure 1. X-ray photograph of the forelimbs and hindlimbs of an arthritic rat that underwent a right cervical rhizotomy (*Rhizo.*) 1 week prior to induction of the arthritis. Both hindpaws show severe swelling and bone destruction (hindlimb score 3; see Materials and Methods). The left forepaw, which has normal innervation, shows mild swelling and injury (forelimb score 1). The forepaw in the rhizotomized right limb, however, shows severe injury (forelimb score 3). Not only is there greater swelling of soft tissue, but the degree of joint destruction is much greater than is typical for forelimbs in nonlesioned arthritic rats.

root. Therefore, to assess the mechanism through which dorsal rhizotomy exacerbated experimental arthritis, we next evaluated the effect of dorsal rhizotomy on the severity of joint injury in rats pretreated with either capsaicin or guanethidine.

The radiographs taken 28 d after injection of mycobacteria revealed that despite an attenuation of the absolute severity of arthritis produced by neonatal treatment with capsaicin, there was still an asymmetric arthritis. That is, dorsal rhizotomy significantly exacerbated the arthritis on the operated side in animals pretreated with capsaicin (see Table 2: 1.6 ± 0.3 vs 0.4 ± 0.2 , $n = 8$; $p < 0.05$ by Wilcoxon paired sample test).

In contrast, in sympathectomized and rhizotomized rats, there was no significant difference in the severity of joint injury of the intact and deafferented limbs (see Table 2: 1.5 ± 0.4 vs 0.8 ± 0.5 , $n = 6$; $p = \text{n.s.}$ by sign test). That is, after sympathectomy, but not after capsaicin treatment, the exacerbation of arthritis seen in the rhizotomized limb was significantly reversed.

Intracerebroventricular administration of morphine

Radiologic examination 28 d after adjuvant injection revealed that the arthritis in the hindpaw of rats treated with intracerebroventricular morphine was significantly less than in rats treated with saline (see Table 3: 0.8 ± 0.2 vs 2.1 ± 0.4 , $n = 20$ and

12; $p < 0.0213$ by Fisher exact test). It was also significantly less than the arthritis seen in normal rats (0.8 ± 0.2 vs 2.4 ± 0.1 , $n = 20$ and 60; $p < 0.001$ by Cochran corrected χ^2 test). No difference between normal rats and those injected with saline in the cerebral ventricles was found (2.4 ± 0.1 vs 2.1 ± 0.4 , $n = 60$ and 12; $p = 1.00$ by Fisher exact test).

Discussion

These studies indicate that the effect of peripheral denervation on the severity of adjuvant-induced arthritis in the rat cannot be ascribed to loss of a particular neural subset but rather reflects changes in the activity of both afferent and efferent components of the PNS. Attenuation of arthritis was produced by administration of capsaicin at birth. The latter observation extends previous results (Colpaert et al., 1983; Courtright and Kuzell, 1965; Levine et al., 1984, 1985) and is consistent with the hypothesis that small-diameter afferents contribute to neurogenic inflammation. A more complete attenuation was, however, produced by sympathectomy prior to injection of the mycobacterium. Arthritis was also more severe in the SHR. These data indicate that the postganglionic innervation of the joint is critically involved in the inflammation associated with arthritis. Finally, the effects of dorsal rhizotomy, alone or in combination with capsaicin or guanethidine pretreatment, indicated that ad-

ditional nerve components, possibly large-diameter sensory afferents, must be involved.

Sympathectomy

Of the various manipulations that attenuated joint injury in arthritic rats, the most profound was sympathectomy, produced with either guanethidine or reserpine. Whereas reserpine depletes catecholamine levels in both the CNS and PNS, guanethidine depletes only peripheral stores, for a period of at least 1 month after its administration is terminated. Thus, although a central effect of these drugs cannot be ruled out, sympathetic denervation of the joint appears to be sufficient to severely impede the inflammatory process that is manifested as arthritis in the rat. The suggestion that activity in the SNS contributes to severity of joint injury is also supported by the finding that SHRs, which have increased activity in their sympathetic nervous system, develop more severe arthritis than their normotensive (WKY) controls.

The effects of guanethidine conceivably could be secondary to its action on the immune system, rather than to the depletion of catecholamines. Specifically, immunologic responses induced by guanethidine may have attenuated the immunologic response to the subsequent injections of the mycobacterium, in such a way that the subsequent arthritic condition was reduced. The studies with reserpine argue against that hypothesis. The effects of reserpine are mediated through depletion of catecholamines. We conclude that the attenuating effects of sympathectomy on arthritis are due to neurotransmitter (presumably norepinephrine) depletion of postganglionic sympathetic joint efferents.

The mechanism through which release of norepinephrine in the joints exacerbates arthritis is not known. Anatomical studies have described sympathetic innervation in the region of the synovial membrane (Halata and Groth, 1976; Langford and Schmidt, 1983). Using the glyoxylic acid method (Loren et al., 1976), we also found catecholamine innervation of the synovial membrane of the rat (J. D. Levine, S. J. Dardick, M. F. Roizen, C. Helms, and A. I. Basbaum, unpublished observations). Most of the norepinephrine varicosities we observed, however, were associated with local vasculature of the joint capsule. Because sympathetic innervation modulates blood flow and vascular permeability, increased activity of the postganglionic sympathetic neurons innervating the joint might exacerbate the arthritis either by increasing access of the immune response to the joint space or by preventing clearance of inflammatory mediators of immunocompetent cells from that joint. It would be particularly relevant to determine whether sympathectomy performed after injection of mycobacterium or after the appearance of clinical signs of arthritis could attenuate the severity of arthritis. To this end, we are now examining the effects of reserpine-induced depletion of catecholamines at various times during the course of arthritis. Our aim is to correlate immunologic events with changes in the activity of the SNS during the development of arthritis.

Treatment with capsaicin at birth

The effect of treatment with capsaicin at birth on the severity of joint damage was consistent with the results from Colpaert et al. (1983). They found that injection of capsaicin into rats before or after the onset of clinically apparent arthritis attenuated the swelling and tenderness produced in response to the inducing antigen. Because capsaicin destroys predominantly small-diameter afferent fibers, the integrity of these afferents is clearly important to the process of joint inflammation. In part, the reduction in inflammation presumably reflects disruption of the normal peptidergic innervation of the joint. We have shown, for example, that increasing the concentration of SP in the knee joint (which is normally at low risk for developing severe arthritic changes) increases swelling and damage to the

Table 3. Degree of joint injury in the hindlimb of rats arthritized after intracerebroventricular injection of morphine or saline

Intracerebroventricular injection	n	Radiographic score ^a (%)					p
		0	1	2	3	Mean	
Morphine	20	55	25	10	10	0.8	<0.022
Saline	12	25	8	17	50	2.1	

^a See Table 1 for explanation.

joint. By depleting the joint of neuronally derived SP, capsaicin would significantly decrease the contribution of the PNS to the inflammation associated with arthritis. Of course, SP is but one of many peptides found in peripheral afferent fibers. It is, however, the most potent inflammatory substance derived from such fibers known today.

Although capsaicin depleted only 63% of the SP in the joint capsule (Levine et al., 1984), this does not imply that a large proportion of SP-containing afferents survived the capsaicin treatment. Neurectomy (i.e., total denervation) depletes only 76% of SP in the skin (Gamse et al., 1980). Apparently there is a non-neuronal SP source, or neuronal SP may be captured and stored by non-neuronal elements after capsaicin or neurectomy. Our results are thus compatible with the observation of others that the doses of capsaicin used in this study destroy approximately 90–95% of unmyelinated afferents (Lawson, 1981; Nagy et al., 1983). The doses of capsaicin used in the present experiments, in fact, almost certainly also damaged small myelinated (A-delta) afferents (Nagy et al., 1983); however, these fibers have not yet been implicated in neurogenic inflammation, and thus their injury is unlikely to explain the attenuating effects of capsaicin on the severity of joint injury in the arthritic rat.

Dorsal rhizotomy

Dorsal rhizotomy was initially included as a control surgical procedure. We wanted to assess the contribution of sensory afferents independently of the somatic and visceral efferents, which also are cut when a peripheral nerve is transected. Rhizotomy also permitted an assessment of the central and peripheral components of small-diameter afferent fibers that were implicated in neurogenic inflammation by the studies on capsaicin. We hypothesized that either the severity of arthritis would decrease after rhizotomy, as occurs after peripheral nerve section or treatment with capsaicin, or no effect would be produced. Had the latter result occurred, it would have implicated the loss of the peripheral terminals of the sensory afferents in the attenuation of arthritis by capsaicin.

We were thus surprised that dorsal rhizotomy markedly increased the severity of joint damage on the deafferented side. The effect was most evident after cervical rhizotomy. In those animals, the severity of the arthritis of the forelimb, which is typically mild in unoperated rats, approached that normally seen in the hindlimbs. Most important, dorsal rhizotomy produced asymmetry in the severity of arthritis in rats given capsaicin at birth. The latter result indicated that a component of the afferent innervation, different from the small-diameter, predominantly unmyelinated afferents, must also contribute to the inflammatory mechanisms underlying experimental arthritis.

Several hypotheses concerning the mechanism(s) through which dorsal rhizotomy exacerbates arthritis can be proposed. Since dorsal rhizotomy does not destroy the dorsal root ganglia, the peripheral afferent innervation of the joint remains. Thus, the peptidergic innervation of the joint persists in rhizotomized rats. The increased arthritis after rhizotomy could therefore have resulted from increased activity of the peripheral afferent joint

innervation. It is certainly likely that dorsal rhizotomy significantly alters the chemical makeup of the dorsal root ganglia and of the distal branches of the cut axons. For example, when the peripheral branch of a sensory afferent is cut, the severed axons sprout rapidly and, if not appropriately guided to innervate target tissue, form a neuroma. Individual axons within the neuroma have an increased spontaneous discharge and become particularly sensitive to catecholamines (Devor and Janig, 1981; Scadding, 1981; Wall and Gutnick, 1974). Conceivably, dorsal rhizotomy leads to development of a neuroma on the cut dorsal rootlets. Enhanced activity of the axons in this proximal neuroma could propagate impulses antidromically (that is, distally) into the peripheral terminals in the joint. If this resulted in increased liberation of neurogenic inflammatory mediators, an exacerbated arthritis might ensue.

On the other hand, those studies that directly examined the effects of dorsal rhizotomy on peripheral histology (Lieberman, 1971) or on SP (Jessell et al., 1979) and fluoride-resistant acid phosphate (Tenser, 1985) levels found no change. In fact, our electrophysiologic analysis of single-unit activity in the saphenous nerve (7–21 d after dorsal rhizotomy) revealed only low levels of spontaneous activity in approximately 4% of the small-diameter C- and A-delta afferents (J. D. Levine, S. J. Dardick, M. F. Roizen, C. Helms, and A. I. Basbaum, unpublished observations). Finally, since capsaicin treatment did not prevent the enhanced inflammatory response produced by dorsal rhizotomy, it appears that factors other than (or probably in addition to) small-diameter axons of the dorsal root must logically be implicated.

It is possible that inflammatory mediators are released from the peripheral terminals of large-diameter afferent fibers. However, we favor the hypothesis that the effects of dorsal rhizotomy reflect central changes that are produced by spinal cord deafferentation, changes that are secondary to the loss of tonic controls exerted by large-diameter fibers (Basbaum and Wall, 1976). Under some conditions, large-diameter myelinated afferents exert inhibitory effects on sympathetic neurons (Koizumi and Brooks, 1972; Sato and Schmidt, 1973). Paralleling this evidence is the fact that nonnoxious inputs (carried predominantly by large-diameter afferents) profoundly inhibit activity in the spinal cord, probably including that of sympathetic preganglionic neurons. For example, transcutaneous electrical nerve stimulation, which selectively activates large-diameter afferents, inhibits sympathetic postganglionic neurons (Abram et al., 1980; Lundberg, 1983). It was particularly significant that sympathectomy counteracted the exacerbation of arthritis produced by dorsal rhizotomy. Taken together, these data strongly support the suggestion that the increased severity of arthritis in limbs subjected to dorsal rhizotomy is mediated, at least in part, by increased release of catecholamines from postganglionic sympathetic neurons, probably through loss of inhibitory controls mediated by large-diameter fibers. As a first step in evaluating the hypothesis that rhizotomy results in increased activity in sympathetic neurons, we have measured the norepinephrine content of peripheral nerves in rhizotomized rats. In fact, the norepinephrine level of the sciatic nerve was found to be significantly higher in rhizotomized than in control rats (3820 ± 685 vs 1960 ± 250 pg/nerve, both $n = 4$, $p < 0.001$ by Student's t test). This result suggests that there is increased synthesis of norepinephrine in sympathetic ganglion neurons after dorsal rhizotomy.

Although the combination of deafferentation and sympathectomy produced no statistically significant differences between the 2 forepaws, there appears to be a trend towards increased severity in the forepaw ipsilateral to the rhizotomy. Thus, we cannot rule out the hypothesis that other factors (including unmyelinated afferent fibers) contribute to the effect of rhizotomy on the severity of joint injury in rats with adjuvant-induced arthritis. To further evaluate the mechanisms under-

lying the asymmetric arthritis produced by dorsal rhizotomy, we are now studying the effect of combined guanethidine and capsaicin treatment in rhizotomized rats.

Central morphine

The conclusion that dorsal rhizotomy increases arthritic severity by removal of inhibitory controls on sympathetic neurons is also consistent with the decrease in the severity of arthritis that was produced by intracerebroventricular injection of morphine. There was no evidence that the regimen of morphine (72 hr, beginning 1 hr before the adjuvant was injected) produced a sustained sedative effect that might have influenced the severity of the arthritis. Rather, it is likely that the morphine disrupted some process that occurs during a critical period between injection of adjuvant and the development of arthritis. There is considerable evidence that morphine, by increasing the activity of brain stem spinal descending control systems, can powerfully decrease the firing of spinal cord neurons (Basbaum and Fields, 1984). The majority of studies have focused on the control of spinal nociceptive neurons by morphine, but there is also profound control exerted on autonomic function (Karoum et al., 1982). In fact, the raphe spinal axons that, in part, mediate descending controls project densely to cells of the intermediolateral column of the thoracic cord, as well as to nociceptive neurons of the spinal dorsal horn (Basbaum et al., 1978).

In conclusion, the present study provides new evidence indicating that the nervous system makes a significant contribution to the inflammatory response of experimental arthritis in rats. The effects of the different nerve lesions indicate that no one class of nerve fiber is wholly responsible for the neurogenic component of the inflammatory response. Changes in the activity of small- and large-diameter sensory afferents and in pre- and postganglionic sympathetic efferents profoundly affect the severity of joint injury. Both the central and peripheral components of sensory afferents, as well as local circuits within the spinal cord, contribute to inflammation. It is unlikely that activity of the PNS directly causes arthritis to develop, but it may influence the "neurochemical milieu" of the joint capsule. The latter may significantly affect the severity, and even persistence, of the existing arthritic disease process.

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